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TECHNIQUES FOR MAINTAINING A CULTURE OF THE BLACK SWALLOWTAIL BUTTERFLY, *PAPILIO POLYXENES ASTERIUS* STOLL (PAPILIONIDAE)

MAUREEN CARTER AND PAUL FEENY

Section of Ecology and Systematics, Cornell University,
Ithaca, New York 14853

ABSTRACT. A culture of the black swallowtail butterfly, *Papilio polyxenes asterius* Stoll (Papilionidae) is initiated from field-collected females. One hundred to two hundred larvae are reared on potted plants in a greenhouse. Adults are housed in an environmental growth chamber with a 16 L/8 D photoperiod, day and night temperatures of 27°C and 15.5°C, respectively, and with a relative humidity at 70 ± 15%. Adults are hand-fed and hand-paired.

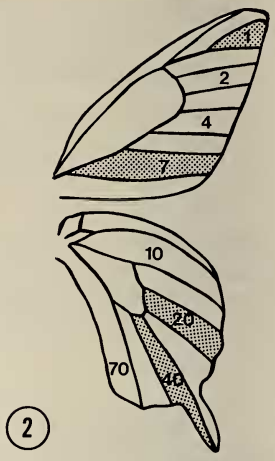
Successful rearing techniques are often a prerequisite for experimental success, yet they are seldom discussed in scientific articles. Our study of the behavior of the black swallowtail butterfly (*Papilio polyxenes asterius* Stoll) has required the maintenance of a year-round culture of this butterfly. Here we describe techniques that may be useful to other researchers who would like to rear this or related butterfly species. We do not claim originality for many of these techniques; some are scattered in the literature, while others have been developed by staff and graduate students in our research group or suggested to us by lepidopterists elsewhere.

Initiating a Culture

Collecting. A stock of wild female butterflies is collected locally. In central New York, peak collecting is generally during the second annual brood, from early July to late August (Lederhouse, 1978). Lederhouse (1981) found only 2.3% of field-caught females to be virgins, and we assume that our wild-collected females have already been fertilized. Captured butterflies are placed in 3½″ square, glazed paper envelopes[1] and transported back to the laboratory in a cool, shady place in the vehicle. In the laboratory, they are housed in a walk-in environmental growth chamber[2] under a 16 L/8 D photoperiod, day and night temperatures of 27°C and 15.5°C, respectively, and at a relative humidity of 70 ± 15%.

Feeding. Butterflies are immediately fed a 10% solution of honey in water, poured into the inverted top of a small petri dish, the inverted bottom of which floats on the solution (Fig. 1). Each butterfly is held close to the dish and its proboscis is unrolled with an insect pin; the

[1] Ward's Natural Science Establishment Inc., P.O. Box 92912, 5100 West Henrietta Road, Rochester, NY 14692-9012.
[2] Environmental Growth Chambers, P.O. Box 407, Chagrin Falls, OH 44022.



FIGS. 1 & 2. **1,** butterflies at honey-water feeding station; **2,** undersides of butterfly wings illustrating markings for number 68.

tip of the proboscis is placed into the honey-water. If feeding occurs, it will continue for 1–5 minutes. This technique allows butterflies to feed with minimal contamination of the legs, abdomens and wings. We have been unable to persuade *P. polyxenes* adults to initiate feeding in the laboratory without assistance.

Numbering. We use a 1-2-4-7 marking system (Ehrlich & Davidson, 1960; Brussard, 1971; Southwood, 1978) as illustrated in Fig. 2. This technique was first used in our black swallowtail culture by Lederhouse (1978). Butterflies can be numbered from 1 to 99; additional numbers are available by changing marker color.

Oviposition. After numbering and feeding, each female butterfly is caged with a potted host plant. Cages are wood-framed, 34 cm × 46 cm, and covered on five sides with organdy mesh or "no-see-um" netting. We do not use wire mesh, because the resistance produced by gripping butterfly tarsi can cause them to break off in handling. Old or worn specimens are placed in plastic shoe boxes[3] lined with paper towels and containing sprigs of host plant fitted with water-filled "Aquapics"®,[4] as shown in Fig. 3. Lids are left ajar or the center is cut out and replaced with wire mesh for ventilation. Because host plant sprigs will desiccate before eggs hatch, the eggs are removed by light nudging with a fingernail and are placed in a plastic petri dish,[5] 15 cm in diameter, lined with moist filter paper.

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[3] Tri-State Molded Plastics, Inc., P.O. Box 6, Dixon, KY 42409.
[4] Cleveland Plant and Flower Co., Wholesale Florists, 262–272 Clinton Street, Binghamton, NY 13905.
[5] VWR Scientific Inc., P.O. Box 1050, Rochester, NY 14603.

FIGS. 3–7. **3,** plastic shoe box with host plant sprigs in "Aquapics"® for oviposition or larval feeding; **4,** individual larval rearing cage; **5,** butterfly emergence cage; **6,** hand-pairing of butterflies; **7,** butterflies *in copula*.

Rearing Immature Stages

We have relied on two methods of rearing larvae: (1) on potted plants, and (2) on excised leaves in closed containers.

Rearing on potted plants. Egg-laden plants are kept on a cart in the greenhouse, under a 16-hour light cycle provided by 400 and 1000 watt metal halide lamps.[6] Egg-laden plants are watered daily. Depending on temperature, eggs hatch in 3–5 days. In the greenhouse, when larvae are present, temperatures are maintained between 24 and 27°C. Automatic misting systems should not be used for watering plants containing larvae younger than the fourth instar, due to high mortality from drowning.

As larvae grow, fresh pots of food plant are placed around them. The larvae will remain on the plants if an ample supply of food is available. Frass is swept out daily. Because larval feeding is minimal

[6] General Electric Company, Hendersonville, NC.

during the first three instars, an abundance of larvae can be maintained. At the fourth instar, the number of larvae is decreased to the number of adults needed, plus 15% to allow for accidental deaths and abnormal specimens. Culture larvae are transferred directly to potted food plants on a greenhouse bench, where they range freely until the end of the fifth instar. If unconfined, they will wander extensively to find a pupation site. To prevent such larval wandering, we confine the late fifth instar larvae with their potted food plants under metal-framed wire cages, 71 cm × 71 cm, or under cylinders of wire with organdy tops that fit over a single pot (Fig. 4). After completing their feeding and voiding their guts, larvae pupate on the roof or walls of the cage and can easily be collected for transfer to emergence cages.

When fresh food plants are available in the field, larvae are reared on potted plants in the greenhouse until the fourth instar and then on excised stems placed in water-filled jars or Erlenmeyer flasks. Food plant is changed every second day, but water is changed daily. Wire cages cover these containers.

Rearing in closed containers. Larvae can be reared in different types of closed containers. Eggs are removed from plants and transferred to petri dishes kept in our growth chamber. With a fine camel-hair brush or a broken boiling stick, newly-hatched first-instar larvae are moved to excised sprigs of food plant fitted with "Aquapics"® (Scriber, 1977). The food plant is placed in a plastic shoe box lined with paper towels. Because of heavy condensation, paper towels must be changed daily and boxes wiped out. Individual larvae can also be reared in small glass petri dishes or plastic containers with tight-fitting lids and moist filter-paper bottoms. Cut leaves are placed in these without any reservoir. The idea is to keep the leaves from drying out without drowning the larvae. Small rearing dishes must be wiped out every 1–2 days. This method is used frequently by researchers feeding weighed or treated leaf material. Food plant in closed containers can be consumed for two days, but water reservoirs must be filled and liners misted daily. A small twig added to the container is a preferred pupation site. Containers are sterilized routinely in 5% sodium hypochlorite solution. Rearing a large number of larvae in closed containers is extremely time-consuming.

Pupae. Larvae pupate on plant stems and pots or the frame or wire of cages. Prepupae shed their ultimate larval skin in 24–48 hours. Prepupae and new pupae are easily damaged. They are removed from pupation sites by misting the silk pad with water and pulling gently at the silk. Pupae are moved to emergence cages in the growth chamber, 5–6 per cage. Each emergence cage is a plastic petri dish, with

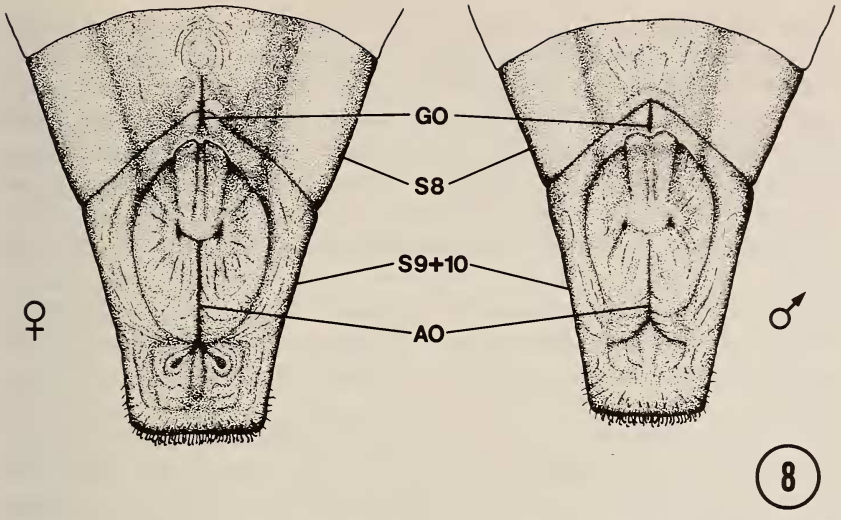

FIG. 8. Abdominal apices of female and male pupae (GO, genital opening; S8, segment 8; S9–10, segments 9 and 10; AO, anal opening).

a "Kimwipe"® liner on the bottom and a 6″ cylinder of wire mesh (Fig. 5).

During June a group of larvae is reared under a short day photoperiod (8 L/16 D) to obtain diapausing pupae. They are stored in a cold environment (0°C) until needed to initiate a new culture, if necessary, during the winter.

Sex of pupae can be accurately determined by close examination of the sutures on the genital plates (Jackson, 1890; Poulton, 1890; Mosher, 1916). The male genital opening is on the mid-ventral surface of segments 9+10 (Fig. 8, GO); while in the female, two genital openings are confluent forming a single slit across the boundaries of segments 8 and 9+10. The genital opening is surrounded by a raised area in both sexes (but more prominently in the female).

Adults

In the laboratory, the adult emergence pattern is protandrous, with eclosion following pupation by 13–20 days (Lederhouse et al., 1982). Eclosing butterflies dry for 24 hours. One day after emergence, each female is marked, fed and bred by the hand-pairing technique developed by Clarke and Sheppard (1956) as illustrated in Fig. 6. Males are fed but not marked. Because Sims (1979) found spermatozoa counts to be low in young males of a closely related species, *Papilio zelicaon* Lucas, males are not mated for 48 hours after eclosing. Each mated

pair of butterflies (Fig. 7) is placed on the inside of a tilted cage. They crawl to the top and hang for 51.3 ± 8.6 minutes (Lederhouse, 1981). Male butterflies can be discarded after the initial mating. If not, they can be remated if rested 48 hours, but spermatophore size will probably be decreased (see Sims, 1979) and copulation time will be longer (Lederhouse, 1981). When the majority of eclosing females have mated, new pots of host plants are introduced to collect 300–400 eggs for the next generation of the culture. Additional heat has been provided to increase oviposition activity. Sixty-watt incandescent or infrared reflector lamps are suspended a minimum of 46 cm above the cages for a period of 3–4 hours. Because egg fertility decreases with time from initial mating (Lederhouse, 1981), we discard mated females after 7–10 days.

During periods of absence, pupae and adults can be stored. Pupae are packed in moist paper towels (see Stone & Midwinter, 1975) and placed in a small insulated cooler. The cooler is stored in a cold environment (0°C) which halts adult emergence. They can stay there for up to two weeks. Adults can be safely refrigerated for 2–3 days. They are put into glazed paper envelopes and placed upright in a cooler after feeding. Butterflies emerging that day are allowed to dry until late afternoon before storage. After removal from the cold environment, they need 2–3 hours to warm up before feeding, the first hour in the envelopes.

Unfortunately, larvae cannot go unattended, even for 24 hours. Potted plants with larvae feeding on them must be watered daily.

Providing Food Plant

Greenhouse plants. Prerequisites for maintaining a culture of this butterfly are a large amount of greenhouse space, readily available food plant in the field, or some combination of the two. We need 325 square feet of greenhouse bench space, supplemented by field-collected plants from June through August, to produce two broods of adults (100–200 individuals each) emerging every six-week period.

Our supply of greenhouse plants for each coming year is started from seed in the spring. Seeds are sown 10–20 per 6″ pot, in sterilized soil or artificial mix. We routinely plant seeds of carrot (*Daucus carota* L.) and parsley (*Petroselinum crispum* (Mill.) Mansfeld). Other food plant species can be planted from commercial or field-collected seeds (Tietz, 1972; Rehr, 1973; Tyler, 1975; Berenbaum, 1978; Scriber & Finke, 1978). Seedlings emerge in two weeks and are thinned before plants are ready for use two months later. Because these plant species are cold weather crops, temperatures in the greenhouse (when larvae

are not present) are kept at 21°C. Evaporative cooling units keep greenhouse temperatures down in the summer.

Plants are routinely watered and fertilized, depending on soil type and climatic conditions. Fertilizing is especially important to promote new growth after the stress of larval feeding. The metal halide lamps, providing a 16-hour photoperiod for larvae, also enhance plant growth in winter. All plants are treated to combat the usual greenhouse pests. We avoid broad-spectrum insecticides and use only those specific for target pest organisms.

After larval feeding, plant stems are cut short to facilitate new growth and provide optimal coverage for pesticide applications. At any one time, ⅓ of our plants are usable for feeding, ⅓ are waiting out twice the residual time of the last pesticide application and ⅓ are just starting to put out new growth. Destruction of some plants by larvae, pruning and pest attacks necessitate having more than one plant per pot. New plants are potted each year, because plants carried over from one year to the next send out flowering stalks and regenerate little new leaf material.

Field-collected leaves. To support larvae in the summer when new greenhouse plants are still maturing, cuttings are collected from wild plants in the field or from plants cultivated in a garden plot. Cuttings are taken in early morning. Cut stems are placed in a bucket of water that is surrounded by ice in a cooler. By taking these precautions, cuttings need only be changed every other day. Cuttings taken during the heat of the day and not adequately protected from dehydration do not remain turgid. New leaves, flower and seed heads are collected. New growth of biennial species can be found in spring and again in late summer.

Morbidity and Mortality

Disease suppression in insects is dependent upon the maintenance of optimal living and rearing conditions (Steinhaus, 1963). These optimal conditions include freedom from stress such as that induced by crowding, toxic chemicals, adverse conditions of light and radiation, inadequate nutrition or lack of oxygen (Steinhaus, 1958, 1963; Burges, 1973). Our rearing method, using potted plants in the greenhouse, provides maximum radiation, air circulation, humidity and space, and optimal food plant quality. Although growth chambers allow more precise control of temperature, humidity and light cycle fluctuations, the incidence of disease in such environments is higher.

Our culture techniques have eliminated epizootic infections to immature stages. However, some enzootic diseases are encountered, and

as Burges (1973) points out most species of leaf-feeding Lepidoptera probably possess a nuclear polyhedrosis virus, a cytoplasmic polyhedrosis virus, a granulosis virus and microsporidians. The small percentage of mortality seen every culture cycle is symptomatic of a fatal bacterial septicemia (Bucher, 1960). Affected larvae discontinue to feed or grow and eventually die; dead larvae hang limp and flaccid, a certain sign of extensive tissue destruction and putrefaction of body contents. The invading bacteria are probably in a class defined by Bucher (1960) as "potential pathogens." These pathogens invade and multiply in the susceptible hemocoele after a variety of stress factors has made the gut more permeable.

No epizootic infections have occurred in the imagos of our culture, although we do see some malformations such as missing or shortened appendages, deformed tarsi, deformed claspers of males, and very small or oversized individuals. One environmental aberration that was once a persistent problem is the condition of a split proboscis. Providing high humidity in the local environment of pupae, either by resting them on a damp substrate (see Stone & Midwinter, 1975) or misting daily (Lederhouse, 1978), can minimize its occurrence. All malformed pupae and adults are eliminated routinely from the culture.

From June to October, prepupae and pupae in the greenhouse must be protected from attack by the hymenopterous parasitoid *Pteromalus puparum* (L.) (Pteromalidae). Cages can be covered with "no-see-um" netting to keep these parasites out.

Maintaining Genetic Variability

Heterozygosity in a domesticated laboratory culture can be lost quickly, especially if the culture is initiated from a few individuals (Benz, 1963). An increase in homozygosity is believed to contribute to a decline in fitness. To maintain genetic variability, we collect many female butterflies from the local wild population to initiate a new culture or add genetic material to an existing one. To avoid inbreeding depression (Mayr, 1970), during the winter months we rear two groups of larvae every six weeks, each with 100–200 individuals. This gives us 50–100 mated females to generate the next cycle.

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